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Olfactory responses of green lacewings, *Chrysoperla* sp. (*carnea* group) and *Mallada desjardinsi* on mealybug, *Phenacoccus solenopsis* (Homoptera: Pseudococcidae) fed on cotton

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Abstract: Host-habitat location of the green lacewing predators, *Chrysoperla* sp. (*carnea* group) and *Mallada desjardinsi* (Navas) (Neuroptera: Chrysopidae) depends on the foraging decisions and behavioral responses towards the plant odors released at various phases. The response of mated adults and mealybug (*Phenacoccus solenopsis*) infesting cotton were studied to understand the tritrophic interactions. Results revealed that both male and female perceived the green leaf volatiles emanating from the cotton plant. All the stages of the plant were responsible for the orientation of the predator and the mealybug. Higher amounts of the saturated hydrocarbons in the infested cotton leaves revealed better responses of the chrysopid adults. The efficacy of the predators can be increased in the fields by releasing the predators at the infested and flowering phase of the plant. Efficient biological control depends on the ability of the natural to establish on the plant and devouring of the pest.

Key words: *Chrysoperla carnea*; *Mallada desjardinsi*; *Phenacoccus solenopsis*; tritrophic interaction; cotton; green leaf volatiles

1 INTRODUCTION

The mealybug, *Phenacoccus solenopsis* (Homoptera: Pseudococcidae) is an important pest of a large number of cash crops (Gautam *et al.*, 2007). Mealybug can be seen throughout the year because of the large range of host plants. Adult females and nymphs are seen often entangled throughout the terminal portions of the plant as white coverings due to presence of waxy coating on their body. The mealy covering affects the penetration of insecticidal spray making pesticide less effective while remains potential for environmental degradation. The pest secretes a large amount of honeydew, which causes the growth of certain fungi causing sooty mould and thus further affects the productivity of the crop. In India, 162 species of insect pests attack different stages of cotton, right from the time of germination till the final picking of cotton bolls (Sundaramurthy and Gahukar, 1998). The decline in the pest status of bollworms due to introduction of Bt cotton, the sap feeders, *viz.*, jassids, aphids, mirids and mealy bugs are emerging as more serious pests (Venilla, 2008). Thus, more advantageous and ecofriendly approaches for controlling the mealybugs are by means of augmentative as well as conservation biological control.

The green lacewings, *Chrysoperla* sp. (*carnea* group) and *Mallada desjardinsi*, well-recognized predators of mealybug in the field which, are widely distributed in India, Europe, USSR, North America, South America, Tanzania, Sudan, Egypt, Kenya and Nigeria. The larva of lacewing is the predatory stage that attacks and feeds on a variety of soft bodied insects like aphids, cicadellids, psyllids, coccids, thrips and also mites (Ridgway and Murphy, 1984; Hagley and Miles, 1987; McEwen *et al.*, 2001, Gautam *et al.*, 2009). Adult lacewings are free living and feed on honeydew and pollen grains although few are predatory (Coppel and Mertins, 1977). The important features of chrysopids include their wide geographic distribution and host range, broad habitats, resistance/tolerance to certain pesticides, good searching ability, voracious larval feeding capacity and easy rearing in the laboratory (Tolstova and Yu, 1986). Moreover,

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inundative release of chrysopid predators has been found to bring immediate and direct reduction in target pest populations (Ridgway and Jones, 1969; Wang and Nordlund, 1994; Singh and Kumar, 2000).

Certain chemicals associated with egg of *Heliothis zea* (Boddie) act as kairomone for *C. carnea* larva that help in prey finding, acceptance or both (Norlund *et al.*, 1997). First and third instar larvae of *C. carnea* are able to distinguish between the odours of the non-prey and prey species and respond significantly to the kairomones of these prey *Acyrthosiphon pisum* (Harris), *Aphis fabae* (Scolopi), *Myzus persicae* (Sulzer) and *Tetranychus urtricae* (Koch) (Sengonca *et al.*, 1995). Ballal *et al.* (1999) studied the host mediated orientation and ovipositional behaviour of *C. carnea*, *Mallada boninenesis* (Okamoto) and *M. astur* (Banks) to cotton, sunflower and pigeon pea in the laboratory. Wind tunnel studies indicated that males of *C. carnea* had a significantly higher preference for sunflower, whereas females for both cotton and sunflower while pigeon pea was the least preferred. Males of *M. desjardinsi* did not show specific preference for any of the three plants, while females preferred cotton. Males and females of *M. astur* did not show specific preference for any of the host plants. The ovipositional patterns followed a similar trend by these species. *C. carnea* has been reported effective in controlling diamond back moth in cabbage (Reddey and Guerrero, 2000) by Integrated Pest Management Program (IPM). Plant species are known to release volatile compounds, referred to as the host-related odours enable many species of natural enemies to locate their prey or prey habitats (Dicke and Sabelis, 1988; Dicke *et al.*, 1990; Paul and Yadav, 2002; Paul, 2003, Yang *et al.*, 2009). Long chain hydrocarbons are commonly encountered in both plant and animal kingdom. In fact n-alkanes are among the commonest constituents of all plant waxes (Baker, 1982; Jeffree, 1986). They play important roles in the trophic level interactions (Bernays and Chapman, 1994). They have been reported to play a role in parasitoid-herbivore (Rutledge, 1996) and predator-herbivore (Yasuda, 1997) interactions, as well as in insect chemical mimicry (Liepert and Dettner, 1996).

In India, cotton occupies an area of nearly 7.39 million hectares; it is the highest consumer of pesticides, followed by rice and vegetables (Patil and Chander, 2008). The most attractive stage of the plant is important for efficient biological control so that the predator gets efficient time for settlement on the crop. Tritrophic interactions play a vital role in the effective use the predator to control the pest. Therefore, the present study was undertaken to investigate the role of chemical cues in the orientation of the predator and prey towards the cotton plant (Sirsa P2) at various stages in laboratory conditions.

2 MATERIALS AND METHODS

2.1 Mass culturing of the predator, *Chrysoperla* sp. (*carnea* group) and *Mallada desjardinsi*

The chrysopids were collected from the cotton fields of the Biological Control Laboratory, Division of Entomology, IARI, New Delhi. They were maintained in the laboratory by using standard procedure set by Gautam (1994b). The predators were identified on the behaviour of the larvae followed by comparison with the laboratory stock. Larvae of *Chrysoperla* sp. (*carnea* group) were reared in acrylic plastic cages having hexagonal cells (25 cm × 25 cm) at 25 ± 2℃, 65% ± 5% RH, and a photoperiod of 12L∶12D (Gautam, 1994). In each cell, single fertile egg of the predator is placed along with frozen eggs of *Corcyra cephalonica* (Stainton) as prey. The prey availability was monitored on alternate days and supplemented based on requirement till larvae reached cocoon formation. The emerging adults were collected daily and kept in the mating cage (25 cm × 25 cm), supplied with a diet (proteinex∶water∶honey∶yeast in the ratio of 1∶1∶1∶1 by volume and 10% honey solution) as food supplement. The adults (8 – 10 day-old) used for the tests were sexed following the morphological characters (Reddy *et al.*, 2004; Gautam *et al.*, 2008).

2.2 Mass culturing of the prey

Mass culturing of the prey insects was done in order to have a continuous supply of the insects for conducting various experiments and to provide food for *Chrysoperla* sp. (*carnea* group) and *M. desjardinsi* larvae. Rearing of mealybug was carried out in glass jars (15 cm × 10 cm) at 25 ± 2℃, 65% ± 5% RH following the method developed by Gautam (2008). Black muslin cloth was used to cover the lid of the jars. Sprouted potato tubers were provided to the mealybugs as food. The jar was checked daily for the mealybug development as well to monitor the contamination. Fresh potatoes were provided to the new generation crawlers for their settlement and healthy colonization so as to get

sufficient population.

2.3 Cultivation of the host-plant

Seeds of *Gossypium hirsutum* collected from Division of Genetics, were grown on the research farm of the Division of Entomology, Indian Agricultural Research Institute, New Delhi in the May – Nov. season. Plant species used in the experiment was cotton (the white gold), *G. hirsutum* also referred to as "New World" species originated in Mexico. The cotton seed of the most popular variety, Sirsa P2 among Haryana farmers was used which is most susceptible to the mealybug infestation. The growers also noticed the variety as susceptible to the mealybug infestation, which helped in the process of selecting this cultivar. The cotton was grown in an area of about 0.25 acres using the standard agronomical practices. The seeds were grown at different plots at an interval of 15 d to harvest the sufficient foliage at various stages of the crop growth. The fresh leaf samples at vegetative phase, flowering phase, infested phase and flower were collected for studies. The cotton plant leaves were utilized for the synomonal extracts. The mealybug biomass was gently collected from the plant during the peak infestation period and utilized for the kairomonal extracts to know the saturated hydrocarbons present.

2.4 Response of mealybug towards the cotton leaves

The leaves were obtained from the plants, grown in the divisional area and free from any pesticide. The experiments were conducted in a room at 25%±2°C, 65%±5% RH with a 40 W fluorescent lamp as a source of light. The room was properly ventilated before carrying out the experiments so that no volatile interferes with the response of the mealybug stages. A glass tunnel of size 14 cm × 2 cm was used for studying the orientational responses. The stimulus was placed at one end of the tube and the other end was left blank. The mealybug responses towards the two ends were recorded continuously for 30 min. The mealybugs reaching the ends of the tube were recorded at the end of the observation period. The tunnel was rotated after each observation so as to avoid any positional error. The observations were replicated ten times; each replicate consisted of 10 individuals (Singh and Mullick, 2002).

2.5 Orientation of mated adults of *Chrysoperla* sp. (*carnea* group) and *M. desjardinsi* to different plant stages

With a view to determining the response of predator towards various stages of the mealybug, each stage was tested separately in a Y-tube of the 20 cm × 20 cm × 20 cm size. Fresh leaves were collected from the plants and carefully wiped with wet cotton and distilled water, without wounding the leaves. The leaves thus were assumed just to release the green leaf volatiles. The petiole of each experimental was wrapped in wet cotton so as to suppress the volatiles emanating from the cut petiole.

The infested leaves for each experiment were carried the mealybug, honeydew and sooty mould. Equal sized leaves were used for all the experiments. The glass tubes were thoroughly washed and then heated to 200℃ after each experiment to make them free from any contamination or sample residue. The position of the Y-tube was continuously changed to compensate for the possible effects due to position, air or light. The individual adult was released at the common end of the tube and allowed to walk up and chose one of the arms. Adults which traveled 8 cm in the arm of Y-tube were considered to make a choice. The glass tubes were thoroughly washed and then heated to 200℃ after each experiment to make them free from any contamination or sample residue. The position of the Y-tube was continuously changed to compensate for the possible effects due to position, air or light. Mean response was calculated and the orientational preference towards the cotton plant and the mealybug (SPSS 15 package). A Chi-square goodness of fit was applied for the individual responses and Chi square test of independent was used to compare the responses of the female and male sex of the two predators that made a choice for a particular odour ($\alpha = 0.05$).

2.6 Gas chromatography

2.6.1 Extraction of synomone: Thirty gram of each host plant leaves were taken at active vegetative phase, flowering phase and infested phase. The leaves were immersed overnight in 300 mL of distilled hexane in separate conical flasks. The hexane was then filtered through Whatman No. 1 filter paper. Anhydrous sodium sulphate @ 1 g/10 g leaves was added to the filterate for dehydration for 2 h and passed through the silica gel (60 – 120 mesh) column. The hexane extract of host plant leaves eluted through the column was then distilled at 60 – 70℃. The left over residue was collected by rinsing the container with little quantity of HPLC grade hexane (Merck) and stored in separate vials in a deep freezer till further use for EAG, GLC-analysis.

2.6.2 Extraction of kairomone: Thirty grams of

the mealy bug infesting the cotton plants were taken and kept in deep freezer for immobilization. The immobilized mealy bug was taken and immersed in 300 mL of HPLC grade n-hexane (Merck AR). The sample was shaken in thermostatically controlled water bath (Haake 220 SWB) at 28℃ for 2 h and later at 50℃ for 20 min at 100 rpm. Rest of the procedure was same as in the case of synomone extraction.

The purified hexane extracts of the plant leaves and mealybug body wash were concentrated and injected into the Gas Chromatograph (Varian 3900 XL) fitted with Flame Ionization Detector (FID) in a WCOT fused silica 30 m (0.32 mm ID, Cp-SIL 24 LB/MS (#CP 5860) Varian Chrompack capillary column at a temperature range programmed between 100 – 260℃ for 56 min. The injector and detector temperature was maintained at 300℃. Nitrogen was used as a carrier gas with a flow rate of 20 mL/min. Injection volume was 3 μL. The hydrocarbon standards (C_{10} – C_{30}) were obtained from Sigma Aldrich, USA. GC of the synomone and kairomones extracts was carried out to detect the presence of these saturated hydrocarbons. The resultant chromatographs were analyzed with the help of interactive graphics (Varian Star Chromatography Workstation, Version 6.0) software. For calculating the quantity of unknown saturated hydrocarbon the following formula was used.

Concentration of unknown saturated hydrocarbon =

$$\frac{\text{Area of unknown saturated hydrocarbon}}{\text{Area of standard saturated hydrocarbon}} \times \text{Concentration of standard saturated hydrocarbon}$$

3 RESULTS

3.1 Orientation of the mealybug towards cotton

The responses of the mealybug stages to the volatiles of freshly excised leaves of different phases of cotton plant under no-choice conditions revealed that the response was highest for the vegetative phase leaves (5.33) followed by infested phase (4.32) and flowering phase leaves (3.35). Orientational preference (%) of the mealybug towards cotton was highest in vegetative phase leaves (53.03) followed by infested phase leaves (37.20) and the flowering phase leaves (27.97) (Table 1 – 3). Responses of the mealybug stages towards the vegetative phase of cotton leaf were in the order of second instar (6.60) > third instar (6.00) > first instar (4.80) > female (3.90), respectively. The response of both second instar and third instar, were statistically significant from female. Mean number of individuals of the first instar, second instar, third instar and female attracted towards the blank end was found to be 1.90, 1.80, 1.70, and 1.10, respectively. Response of the mealybug stages towards the flowering phase leaves showed that highest response was of 3rd instar (4.10) as compared to 2.80 in 1st instar as well as female (Table 2). The response of both 1st instar and 3rd instar, were significantly different from female. Mean response of the 1st instar, 2nd instar, 3rd instar and female towards the blank end was found to be 6.40, 5.00, 2.30 and 1.70, respectively (Table 2). Response of the various mealybug stages towards the infested phase leaves showed that highest response was 2nd instar (6.10) followed by 3rd instar (5.70), female (2.80) and 1st instar (2.70). The response of 1st instar – 2nd instar, 1st instar – 3rd instar, 2nd instar – females and 3rd instar – females were significantly different from each other. Mean response of the 1st instar, 2nd instar, 3rd instar and female towards the blank end was found to be 5.10, 2.90, 2.30 and 1.20, respectively (Table 3).

3.2 Olfactory responses of adults to test plant leaves

Both male and female of *Chrysoperla* sp. (*carnea* group) showed similar responses towards the vegetative and flowering leaves of the cotton plant ($\chi^2 = 9.68$, $P < 0.005$) and ($\chi^2 = 0.32$, $P < 0.005$) respectively. Compared with the clean air the adults were not significantly attracted towards the

Table 1 Orientation of mealybug to the cotton plant at vegetative phase leaves

Mealybug stage	Mean response towards vegetative phase leaf	Mean response towards blank end	Orientational preference (%)
1st instar nymph	4.80 ± 0.35 bc	1.90 ± 0.23 a	43.20
2nd instar nymph	6.60 ± 0.47 a	1.80 ± 0.29 a	57.14
3rd instar nymph	6.00 ± 0.44 ab	1.70 ± 0.33 a	55.80
Female	3.90 ± 0.56 c	1.10 ± 0.27 a	56.00
Mean	5.33 ± 0.28	1.62 ± 0.14	53.03 ± 3.29

Mean of 10 replications. Means within a column followed by different letters are significantly different, $P < 0.05$; repeated measure ANOVA followed by Turkey's test. The same below.

Table 2 Orientation of mealybug to the cotton plant at flowering phase leaves

Mealybug stage	Mean response towards flowering phase leaf	Mean response towards blank end	Orientational preference (%)
1st instar nymph	2.80 ±0.32 b	6.40 ±0.29 a	39.13
2nd instar nymph	3.70 ±0.21 ab	5.00 ±0.33 b	14.94
3rd instar nymph	4.10 ±0.19 a	2.30 ±0.30 c	33.33
Female	2.80 ±0.79 b	1.70 ±0.67 c	24.44
Mean	3.35 ±0.32	3.85 ±1.11	27.97 ±5.29

Table 3 Orientation of mealybug to the cotton leaves at its infestation phase

Mealybug stage	Mean response towards infested phase leaf	Mean response towards blank end	Orientational preference (%)
1st instar nymph	2.70 ±0.33 b	5.10 ±0.40 a	30.76
2nd instar nymph	6.10 ±0.34 a	2.90 ±0.48 b	35.55
3rd instar nymph	5.70 ±0.33 a	2.30 ±0.21 bc	42.50
Female	2.80 ±1.03 b	1.20 ±0.38 c	40.00
Mean	4.32 ±0.91	2.87 ±0.82	37.20 ±2.58

infested leaves for male and female ($\chi^2 = 0.08$, $P < 0.005$) and ($\chi^2 = 2$, $P < 0.005$), respectively. The responses of the adults were significantly greater for the flower ($\chi^2 = 3.90$, $P < 0.005$) and ($\chi^2 = 11.52$, $P < 0.005$) for males and females, respectively. The responses of male and female *Chrysoperla* sp. (*carnea* group) towards the mealybug itself were found to be similar ($\chi^2 = 0.32$, $P < 0.005$) (Fig. 1 and 2). The response of *M. desjardinsi* females was not significant for vegetative leaves ($\chi^2 = 0.32$, $P < 0.005$). The results observed were highly significant for males in comparison to females ($\chi^2 = 6.48$, $P < 0.005$). Comparing the flowering phase leaves to the adults at one end of the Y-tube, it was observed that results were statistically highly significant ($\chi^2 = 8$, $P < 0.005$). The results observed for the male *M. desjardinsi* was found to be highly significant statistically for infested leaves ($\chi^2 = 13.52$, $P < 0.005$). Olfactory responses of the females were not so distinguished ($\chi^2 = 0.72$, $P < 0.005$) when the flower of the cotton plant was used as stimulus source the responses were similar for the male and female ($\chi^2 = 0.068$, $P < 0.005$) and also were not significant. The responses of male and female *M. desjardinsi* towards the mealybug used as visual and volatile stimulus were also not found to be significant ($\chi^2 = 0.72$, $P < 0.005$) and ($\chi^2 = 0.08$, $P < 0.005$), respectively (Fig. 3 and 4). When the data was analyzed for Chi-square test of independent to know better predator the values obtained for the females were vegetative phase leaves: $\chi^2 = 5.95$, $P = 0.15$; flowering phase leaves: $\chi^2 = 2.08$, $P = 0.15$; infested phase leaves: $\chi^2 = 2.25$, $P = 0.13$; flower: $\chi^2 = 9.01$, $P = 0.003$; and mealybug: $\chi^2 = 0.16$, $P = 0.69$. Comparing the data using Chi square test of independence for the males of both species, the results obtained were as follows: vegetative phase leaves: $\chi^2 = 0.048$, $P = 0.83$; flowering phase leaves: $\chi^2 = 2.08$, $P = 0.15$; infested phase leaves: $\chi^2 = 0.36$, $P = 0.55$; flower: $\chi^2 = 6.05$, $P = 0.014$; mealybug: $\chi^2 = 0.16$, $P = 0.69$ (Table 4).

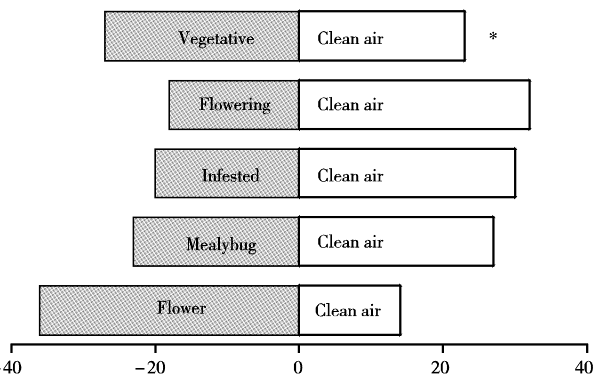

Fig. 1 Olfactory responses of *Chrysoperla* sp. (*carnea* group) females to cotton plant and live mealybug

Asterisk indicates significant difference ($P < 0.05$); n = 50 predators.

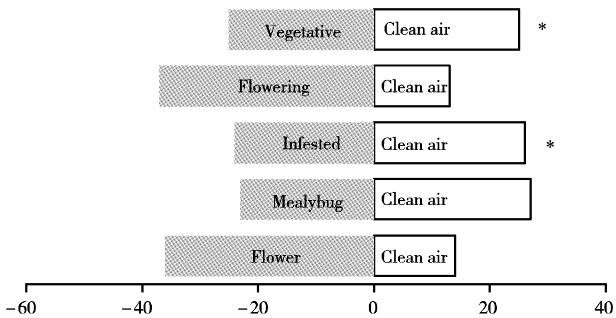

Fig. 2 Olfactory responses of *Chrysoperla* sp. (*carnea* group) males to cotton plant and live mealybug

Asterisk indicates significant difference ($P < 0.05$); n = 50 predators.

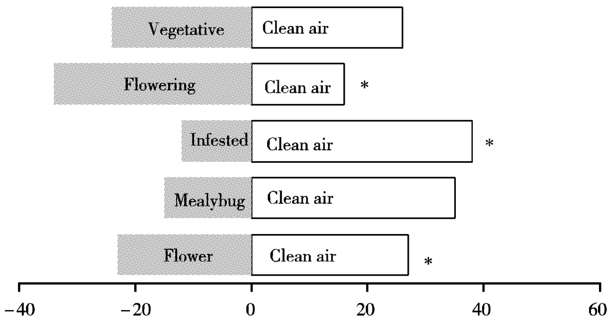

Fig. 3 Olfactory responses of *Mallada desjardinsi* females to cotton plant and live mealybug

Asterisk indicates significant difference ($P < 0.05$); n = 50 predators.

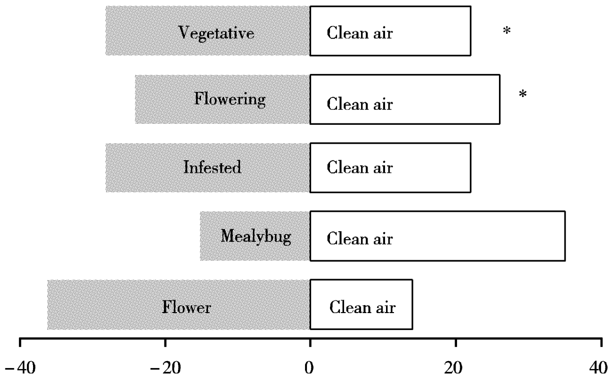

Fig. 4 Olfactory responses of *Mallada desjardinsi* males to cotton plant and live mealybug

Asterisk indicates significant difference ($P < 0.05$); n = 50 predators.

Table 4 Responses of male and female adults of *chrysoperla* sp. (*carnea* group) and *Mallada desjardinsi* to cotton and mealybug

Stages	Males		Females	
	χ^2	P	χ^2	P
Vegetative phase leaves	0.048	0.83	5.95	0.015
Flowering phase leaves	2.08	0.15	2.08	0.15
Infested phase leaves	0.36	0.55	2.25	0.13
Flower	0.16	0.68	0.16	0.68
Mealybug	6.05	0.014	9.014	0.003

3.3 Gas chromatography

Gas chromatography of the vegetative phase leaves clearly revealed the presence of three hydrocarbons ranging from C_{25} – C_{29}. Among the identified hydrocarbons heptacosane (6 407.52 mg/L) was present at the highest concentration followed by nonacosane (1 403.76 mg/L)). The order of the hydrocarbons detected in the vegetative leaf extract based on the quantity was heptacosane > nonacosane > pentacosane. Flowering phase leaves indicated the presence of four hydrocarbons namely: Nonacosane > hexacosane > pentacosane > octacosane. Eighteen saturated hydrocarbons ranging from C_{11} – C_{30} were detected in the infested phase leaves. Among the detected hydrocarbons nonadecane was found to be in the highest concentration (782 237.39 mg/L)) followed by henicosane. The flower revealed the presence of fifteen saturated hydrocarbons: Hexacosane > nonacosane > heptacosane > pentadecane > tridecane > tetradecane > hexadecane > henicosane > nonadecane > octacosane > eicosane > tricosane > tricotane > pentacosane > dodecane. Seventeen saturated hydrocarbons were revealed from the kairomonal extract of the mealybug ranging from C_{13} – C_{30} (Table 5).

4 DISCUSSION

The mealybug *P. solenopsis* is an important pest of more than 250 cultivated plants belonging to at least 80 families. There is a lack of sufficient information about the natural control of this pest which makes this pest of great deal to control. Mealybug is highly mobile in the young stages and becomes sessile as it ages. Young nymphs disperse to find the suitable site and then begin to suck the sap. They can easily be transported with water or wind. Maximum orientation and attraction (53% ± 3.29%) of the mealy bug crawlers towards the cotton plant at infested flowering phase (27% ± 97%) was observed as against minimum (37% ± 2.58%) at vegetative phase. On the other hand, maximum response for the vegetative, flowering and infested phase was shown by 2nd instar (6.60 ± 0.47) (Table 1), 3rd instar (4.10 ± 0.19) (Table 2) and 2nd instar nymphs (6.10 ± 0.34) (Table 3), respectively. It may be referred that infestation at flowering phase attracted both crawlers and 2nd instar nymphs of *P. solenopsis* for colonization on the terminals of the plant while 3rd instar had potential for spread and colonization on leaf foliage. Several studies have been undertaken to know the response of predators towards the plants, prey and host-plant complex. However, Ru and Makosso (2001) stated that the emission of volatile chemicals did not appear to be limited to the infested parts of the plant but did occur systematically throughout the plant. Our results were found to be similar because all the stages of the mealybug showed orientation towards the vegetative, flowering phase and infested leaves of the cotton thought the response for each stage of mealybug was different. The study is of prime importance as it tells us the most attractive stage of cotton plant, which guides the mealybug towards itself.

Table 5 Gas chromatographic analysis of cotton Sirsa P2 at different stages

Saturated hydrocarbons	Vegetative leaves (mg/L)	Flowering leaves (mg/L)	Infested leaves (mg/L)	Flower (mg/L)	*Phenacoccus solenopsis* (mg/L)
Decane	ND	ND	ND	727.80	ND
Undecane	ND	ND	28 387.70	4 746.06	ND
Dodecane	ND	ND	576.80	4 731.48	ND
Tridecane	ND	ND	67 678.12	6 045.85	1 966.10
Tetradecane	ND	ND	26 541.70	4 332.76	2 138.27
Pentadecane	ND	ND	ND	3 276.02	1 931.57
Hexadecane	ND	ND	3 220.90	2 210.43	3 322.51
Heptadecane	ND	ND	ND	3 524.27	5 163.45
Octadecane	ND	ND	5 194.61	727. 80	4 390.31
Nonadecane	ND	ND	782 237.40	4 746.06	18 366.76
Eicosane	ND	ND	232 706.70	4 731.47	12 884.98
Heneicosane	ND	ND	769 817.75	6 045.85	5 851.58
Docosane	ND	ND	302 659.93	ND	1 966.10
Tricosane	ND	ND	16.71	2 019.60	11 694.90
Tetracosane	ND	ND	140 563.36	ND	972.10
Pentacosane	732	774.32	104 958.95	1 765.51	32 849.32
Hexacosane	ND	2 706.76	568 793.02	312 072	30 068.19
Heptacosane	6 407.52	ND	411 841.63	31 459.78	81 521.53
Octacosane	ND	521.86	381 214.24	2 573.45	17 874.56
Nonacosane	1 403.75	3 126.83	232 440.14	124 244.06	29 679.95
Triacontane	ND	ND	467 560.42	1 860.77	24 277.41

ND: Not detected.

Responses of the predator adults towards the various cotton growing phases showed that *C. carnea* easily perceived plant emitted volatiles and corroborates the earlier reports that the natural enemy initially seeks an environment and can recognize host plant factors, irrespective of presence or absence of the prey (Ananthakrishnan, 1992). The responses of adults towards the flowers in the present study might be due to their colour preference observed in *Chrysoperla* sp. (*carnea* group). Maredia *et al.* (1994) reported that the *C. carnea* had a positive response towards colors; the predator had a preference for yellow, green and red. Plants defend themselves by emitting green leaf volatiles (GLVs) in response to herbivore attack (Dicke *et al.*, 1990; Turlings *et al.*, 1990). These GLVs benefit the plant by attracting natural enemies of the herbivores that feed on its foliage and benefits parasitoids and predators by guiding them to potential hosts or prey on the plant (Dicke and Sabellis, 1988; Turlings *et al.*, 1991, 1995; Rose *et al.*, 1998). The females of *C. carnea* used olfactory stimuli and were able to discriminate the chemical stimuli and its concentration, resulting in enhanced search activity.

The lacewings have been known to be associated with a large number of synomonal and synthetic compounds which affect their orientations (Hagen *et al.*, 1976; Emden and Hagen, 1976; Ben-saad and Bishop 1976a, 1976b; Liber and Niccoli 1988; McEwen *et al.*, 1993, 1994; Zhu *et al.*, 1999, 2005; Han *et al.*, 2002; Raina *et al.*, 2004). In the present study the association of the *Chrysoperla* sp. (*carnea* group) with the cotton plant was established and results are in conformity with the findings of Ballal *et al.* (1999) who studied the host mediated orientation and ovipositional behaviors of *C. carnea*, *M. boninenesis* and *M. astur* to cotton, sunflower and pigeon pea in the laboratory.

The response of both male and female *Chrysoperla* sp. (*carnea* group) was maximum for

the infested phase leaves in the free choice test. In the Y-tube experiments more number of male was found in the infested phase of the leaves followed by flowering phase leaves, vegetative phase leaves and mealybug. The higher response of the male towards the infested phase leaves could be attributed to the better chances of finding a mate. The females perceived the volatiles from long distances and preferred to lay eggs where the chances of survival are more *i. e.* to find the best oviposition site. These preferences of the cotton plant could be due to certain chemical cues possibly involved in the acceptance or rejection or the potential hosts.

Also, Reddy (2002) showed that *C. carnea* elicited a positive behavioural response in an olfactometer for the volatiles emitted by eggplant (*Solanum melongena* L.), okra (*Abelmoschus esculentus* L.), and pepper (*Capsicum annum* L.) that are damaged by spider mites (*Tetranchus ludeni* Zacher) whereas no response was observed for the volatiles emitted from tomato (*Lycopersicum esculentum* Mill.). This confirms that *C. carnea* has its own preference for certain plants and no preference for some.

The presence of the saturated hydrocarbons was highest in the mealybug-infested leaves of the cotton. Through gas chromatography, it was confirmed that the response was due to the presence of higher number of saturated hydrocarbons. The maximum number was found in the mealybug infested leaves of the cotton plant (eighteen hydrocarbons) followed by the mealy bug body wash hexane extract (seventeen hydrocarbons) and the number of the saturated hydrocarbons in the flowering and vegetative leaves extract were four and three hydrocarbons, respectively. Among the various stages used the maximum response was obtained from infested leaves followed by *P. solenopsis*, flowering leaves and vegetative leaves. Gas chromatography revealed the presence of three, four, eighteen and seventeen saturated hydrocarbons in vegetative, flowering, infested and mealybug extract respectively.

It was observed that infestation at flowering phase attracted both crawlers and 2nd instar nymphs of *P. solenopsis* for colonization on the terminals of the plant while 3rd instar had potential to spread and colonize on cotton leaf foliage. Rapusas *et al.* (1997) studied the orientation of *Cyrtorhinus lividipennis* Reutertowards volatiles of certain rice genotypes. However, the predator distinguished between prey-infested and uninfested plants and preferred plants with eggs over plants with nymphs. The predator did not distinguish at different stages of plant growth (vegetative, booting or flowering). Plants artificially injured to stimulate brown plant hopper oviposition wounds were not as attractive to the predator as plants on which the plant hopper had oviposited. These results support our findings that the predator responded but not with significant difference towards the different phases of cotton. The predator adults are free-living in nature and feed on honeydew, therefore, they show better response to infested phase and flower. Balakrishnan *et al.* (2006) studied the influence of cotton genotypes on the oviposition preference and predation of *C. carnea* and found negative relationship between the trichome density and the predation of *C. carnea.* Adults feeding on honeydew and pollen grains could be attributed to their better response for the infested phase leaves and the flower stages than the vegetative phase leaves and the flowering phase leaves. The actual predator is the larva but still adults have the ability to locate the host-plant and the prey species for the betterment of the future generations. Volatiles from the cotton plants are general plant volatiles belonging to hydrocarbons including a few green leaf volatiles (GLVs).

Green leaf volatiles contain up to 100 different hydrocarbons (Nelson *et al.*, 1981). The length of hydrocarbons chains usually varies from 23 to 47 carbon atoms (Blomquist and Dillwith, 1985). The chain length of the hydrocarbon in synomonal extracts ranged from C14 – C30. Tricosane acts as the most active compound to elicit a high response in the egg parasitoid *Trichogramma evanescences* (Jones *et al.*, 1973). Hydrocarbons with n-C27 (nonacosane) and nC31 (hentriacontane) are most abundant in apple leaves (Hellmann and Stoesser, 1992). Odours are very important in the behavioural interactions, both attractants and deterrents play an active role in chemical ecology (Renwick, 1989). Preferred hydrocarbons *viz.*, tetracosane, pentacosane and dotricontane at 199.77 mg/L and n-nonacosane at 499.42 mg/L may be used for enhancing the activity of the predator in the natural ecosystem as well as in the release program (Singh and Paul, 2002) which found better in the infested leaves. The main

factor with respect to plant waxes that is thought to determine the foraging success of predators or parasitoids is their ability to locate the plant surface. Hydrocarbons with nC27 (nonacosane) and nC31 (hentriacontane) were reported as the most abundant in plants (Hellmann and Stoesser, 1992), which has been further confirmed by our results. These compounds are known to constitute the texture of the epicuticular leaf surface (Baker, 1982). The amount and composition of alkanes in apple leaves change depending on the season, developmental age of the leaves, and on apple tree varieties (Hellmann and Stoesser, 1992), and similar results were obtained which showed different quantity and numbers of the saturated hydrocarbons in the cotton plant at different phases. *Chrysopa scelestis* showed increased predation in the cotton cultivars having caryophellene (Annadurai *et al.*, 1992). Perception of the odors depends on the antennal receptors, which are compound specific as shown by Zhu *et al.*, 2000 in *C. carnea* the presence of receptor neurons specifically responding to tridecene. Receptor neurons respond selectively to one or a few compounds within the limited range of compounds tested by Anderson *et al.*, 1993. The antennae are equipped with a large number of hairs-like sensillae. These sensila are believed to contain receptor neurons that respond to plant odors (Ljungberg *et al.*, 1993). Receptor neurons have been found to respond to oviposition deterring compounds on cotton (Anderson *et al.*, 1993).

It is suggested that the efficacy of the predators can be enhanced in the fields by releasing these predators at the infested and the flowering phase of cotton crop to curb the high-density pest population. Identification of saturated hydrocarbons in the range of C10 – C30 have the potential for their manipulation in better understanding of the tritrophic interactions as well as developing cultivars to suit the natural enemies. Besides, these synthetic hydrocarbons may be applied in the field to enhance predator's activity.

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两种草蛉对取食棉花的扶桑绵粉蚧的嗅觉反应

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摘要：捕食性的普通草蛉 *Chrysoperla* sp. (*carnea* group) 和安平草蛉 *Mallada desjardinsi* (Navas)(脉翅目：草蛉科)的寄主-生境定位取决于其觅食行为以及对各阶段释放的植物气味的行为反应。本研究调查了这两种交配的草蛉成虫以及取食棉花的扶桑绵粉蚧 *Phenacoccus solenopsis* 的反应，以便了解它们之间的三重营养关系。结果表明：草蛉雄虫和雌虫均能感受到棉花植株释放的绿叶挥发物；植株的各个阶段均可使捕食性昆虫和绵粉蚧定位。在受绵粉蚧为害的棉叶上饱和烃含量高，说明草蛉成虫的反应更为强烈；在植株受为害期和开花期释放捕食性昆虫，可提高田间捕食性昆虫的作用。这些结果提示有效的生物防治取决于捕食性昆虫在植株上的天然定殖能力以及对害虫的吞食能力。

关键词：普通草蛉；安平草蛉；扶桑绵粉蚧；三重营养关系；棉花；绿叶挥发物

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